With the author's best rega

(7)

ON THE

CHEMICAL COMPOSITION

OF THE

NUCLEI OF BLOOD CORPUSCLES.

BY T. LAUDER BRUNTON, B.Sc., M.D.,

LATE SENIOR PRESIDENT OF THE ROYAL MEDICAL SOCIETY OF EDINBURGH.

[*Reprinted from "The Journal of Anatomy and Physiology" for November*, 1869.]

DURING the course of last summer Professor Kühne discovered that the chief constituent of the Nuclei of Blood Corpuscles agreed in its reactions with mucin rather than fibrin or albumen. It had previously been found by Hoppe Seyler,* associated in the nuclei with a small amount of paraglobulin, and previous to Professor Kühne's discovery, had been supposed to be an albuminous substance, resembling fibrin. I was informed by Professor Kühne, while working in his laboratory in Amsterdam, of the observations he had already made, and having repeated them, I publish the result with his permission. The observations are not complete, but I give them now, as I am unable to prosecute them further at present.

The nuclei of the blood corpuscles of eels and frogs yield a substance similar to that obtained from the blood of fowls; but as the latter could be much more readily obtained in considerable quantity, it alone was used in studying the reactions in detail.

To obtain the nuclei, the defibrinated blood, mixed with ten

* Kühne, Lehrbuch der Physiologischen Chemie.

or twelve times its volume of NaCl solution of 3 per cent. is filtered through linen, and the corpuscles allowed to subside in a flat tray. The supernatant fluid is then removed by a syphon, and the corpuscles, thus freed from serum, are either washed repeatedly with much water in the same manner, or after being allowed to settle in the salt solution for at least twenty-four hours, when they form a kind of film, are scraped together, and washed on a linen filter. In the former case, the nuclei or rather zooids of the blood corpuscles are obtained as a white powder, which sinks very slowly in water; in the latter, as a mass resembling fibrin in appearance. Microscopic examination shows this powder to consist of the nuclei in the form of small round bodies containing several dark granules, surrounded by a ring of transparent colourless substance, apparently a remnant of stroma, whose breadth is about equal to the diameter of the nucleus, and whose edge is so delicate as to be scarcely perceptible. On the addition of aniline, red or blue, dissolved in dilute alcohol, the nucleus becomes deeply coloured, the stroma slightly so, and its edges much more distinct. Weakly alkaline solutions of carmine and solutions of iodine also colour the nucleus deeply, but the stroma very slightly, or not at all. The nucleus is generally in the middle, but, occasionally, is more or less eccentric, and sometimes sticks quite close to one side of the surrounding substance. This last may possibly be its constant situation, and its central one only apparent, and it may thus correspond to the point in mammalian blood corpuscles, which was found by Roberts* and Rindfleisch† to become deeply coloured by magenta. If the powder be then shaken with ether and water it forms a layer between the two; and when this is microscopically examined, the nuclei alone are seen, the stroma formerly surrounding them being no longer perceptible even after the addition of aniline. The nuclei may be got at once by treating the corpuscles with ether, separating the nuclear layer by a stoppered funnel, and then washing in water. Alkalis cause the nuclei to swell, to run together in clumps,

* Proceedings of Royal Society, 1863.

† Experimental studien über die Histologie des Blutes.

become indistinct, and finally disappear. Dilute mineral acids or acetic acid cause them to shrink and become more sharply defined. A small, strongly refracting point, resembling a nucleolus, and seeming to take up the colouring matter more strongly than the rest, also becomes visible; but this appearance may be due to a change of shape in the nucleus, occasioned by the acid. Concentrated mineral acids cause them to shrink much, to run together, become indistinct and disappear. The stroma surrounding the nuclei swells and shrinks somewhat, but not so markedly as the nuclei. If ferrocyanide of potassium be added to the nuclei shrivelled by acetic acid they swell up, and become so indistinct as to be hardly visible. A solution of taurocholate or glycocholate of soda dissolves both nuclei and stroma. A little concentrated NaCl solution also causes the nuclei to disappear. When the corpuscles are washed on a linen filter, a fibrinous-looking mass is obtained, which, on microscopic examination, is seen to consist of shreds of fibrous membrane, or of bundles of fibres, studded with darker spots, and arranged in a manner resembling those of fibrin, though more regular and with less intercrossing. These spots seem to be the nuclei, but their outline is not so distinct, nor do they take the deep tint with aniline which they do in the powdery condition, the fibres becoming quite as deeply tinted as they.

The zooids are insoluble in water, and when suspended in it sink very slowly, but do so much more quickly after the addition of alcohol, concentrated acetic or oxalic acid, or dilute mineral acids. The mixture with water is quite mobile, and does not foam when shaken; but does so after the addition of a little NaCl solution, becoming at the same time somewhat tenacious and much clearer, the nuclei being partly dissolved and partly suspended. A concentrated mixture with NaCl solution gives a white flocky precipitate when much diluted. Salt solutions, of even one-fourth per cent., dissolve them to a considerable extent. The solubility in NaCl solution varies much, diminishing when the zooids stay long in water, but more slowly when the temperature is low. The same is the case with mucin obtained from tendons. When many zooids are

suspended in water, one drop of concentrated solution of potash or soda is sometimes sufficient to convert 40 cubic centimetres of the mixture from a milky mobile liquid to a clear gelatinous mass, resembling albuminate of potash in appearance, though not quite so firm. When this is thrown on a filter, the filtrate gives no precipitate with acetic acid. When more potash is added, a tenacious ropy fluid is produced, which filters very slowly, the filtrate is mobile, and though generally more or less alkaline, is sometimes neutral. Alkaline carbonates dissolve them, but much more slowly, nor do they form a jelly like the caustic alkalis; sometimes, however, they cause the zooids to stick together and form flocks, which, rising to the top, form a sticky mass. Lime and baryta water leave them apparently unchanged, and after standing on them some time, give no precipitate with acetic acid, but an immediate turbidity if ferrocyanide of potassium be then added. Concentrated mineral acids dissolve the zooids, and give a precipitate on the addition of alkalis or much water.

Dilute mineral acids, such as HCl of 10 per cent., cause the mixture with water to foam on shaking, but when the filtered fluid is made alkaline by potash it gives no precipitate with acetic acid but a turbidity, when ferrocyanide of potassium is then added. The filtered solutions of the zooids in alkalis give the reactions of albumen, but the precipitate by acetic acid is generally insoluble in excess. Sometimes, however, not only the mucin from nuclei, but that from glands and tendons, appears quite soluble in large excess of glacial acetic acid. If the zooids be treated with ClH of $\frac{1}{10}$th per cent., or acetic acid or NaCl solution of 10 per cent., and the filtered solution be precipitated by acetic acid and again filtered, the clear fluid in each case gives the reactions of albumen. The HCl solution is precipitated on neutralization, and the precipitate is insoluble in NaCl solutions of 10 per cent. The albuminous body thus belongs to that class which includes, according to Hoppe Seyler, fibrinogen, fibrinoplastic substance, and myosin. That the zooids contain fibrinoplastic substance or paraglobulin, as stated by Hoppe Seyler, is shown by the distinct fibrinoplastic action

which they exert when well washed. Sometimes they possess none at all; and this is probably due to the removal of the substance in the washing, the salt solution with which the corpuscles were washed not having been sufficiently carefully removed, and rendering the first water a dilute salt solution, which dissolves a certain amount both of albuminous substance and of mucin, becomes milky after standing or passing CO_2 through it, and possesses a slight fibrinoplastic effect. The fibrinoplastic effect was tried in all cases with a mixture of horse plasma and sulphate of magnesia. From the way in which fibres are formed when the zooids are washed on a linen filter, it seems probable that fibrinogenic substance may also be present; but whether this be the same as mucin, or what the relation between mucin and the generators of fibrin or myosin, if any such relation exists, is still to be investigated. When the precipitate from solutions in alkalis or NaCl by acetic acid is washed with acetic acid, then with dilute alcohol, and afterwards dissolved in a *small* quantity of potash and filtered, the filtrate is generally alkaline, but sometimes neutral. It is unchanged by boiling, gives with mineral acids a precipitate soluble in excess, and with acetic acid a precipitate insoluble in excess. On exceptional occasions, I have seen it, as well as mucin from tendons dissolved by excess of glacial acetic acid, give with acetic acid and ferrocyanide of potassium no turbidity, the ferrocyanide of potassium causing any turbidity from the acetic acid to become less and disappear; but after standing a considerable time a precipitate forms. Chloride of mercury causes no precipitate; with tannin acetate of lead or dilute sulphate of copper or chloride of iron it gives a precipitate. Added to potash and sulphate of copper it prevents the precipitation of the hydrated oxide of copper, but the solution remains blue even after boiling.

Nuclei freed from stroma by ether and water and then dissolved in potash give the same reactions. These reactions differ from those of mucin as given by Eichwald, (Kühne, *Lehrbuch der Physiologischen Chemie*,) inasmuch as tannin, sulphate of copper and chloride of iron give a slight precipitate or

turbidity, but on treating nuclei and mucin from glands and tendons in the same way they give the same reactions. When a salivary gland is treated by potash, and the solution precipitated by acetic acid, the precipitate is sticky, and seems to differ much from that given by acetic acid in solutions of nuclei in potash, which is flocky, and gathers on a linen filter into a mass, looking like boiled fibrin ; but if the strongly acid and sticky precipitate from the gland be allowed to stand some time in water, it becomes exactly like that obtained from the nuclei. The zooids and their solution in NaCl act briskly on peroxide of hydrogen ; the nuclei after treatment by ether and water do so also but less vigorously. When boiled with dilute sulphuric acid they gave no trace of sugar. I have never succeeded in obtaining them free from sulphur even after repeatedly dissolving in potash, and precipitating and washing by acetic acid; but the more carefully they were cleaned the less sulphur was found ; and Professor Kühne on one occasion obtained no trace of sulphur after burning with nitrate of potash and adding chloride of barium. This trace of sulphur may possibly depend on a little albumen carried down with the mucin ; more especially as one sees that if the, homoglobin be not entirely removed by washing before dissolving in potash, and precipitating by acetic acid, hæmatin is constantly carried down with the precipitate, and cannot again be separated. When chicken blood is treated by NaCl solution of 10 per cent., as in Professor Heynsius' experiments, lately published, the nuclei are dissolved, and form a large portion of the fibrinous-looking substance he describes. Whether mucin exists in mammalian blood or not I cannot certainly say, though the substance got by treating dogs' blood with salt solution of 10 per cent., and then washing the slimy mass, seemed, after solution in potash, to give a precipitate with acetic acid insoluble in excess. The quantity obtained pure was however so small that I was unable to try any other reaction.

Shortly then, the substance of the nuclei, both with and without the stroma, agrees with mucin, and differs from albumen in its insolubility in HCl of 0·1 to 1 per cent., in its

alkaline solutions being precipitated by nitric, hydrochloric or sulphuric acid, and the precipitate dissolved without difficulty by excess; in being precipitated by acetic acid, and the precipitate insoluble in excess, ferrocyanide of potassium causing no further turbidity, but clearing up any formed by the acetic acid; in neutral solutions being unchanged by boiling, and giving no precipitate with chloride of mercury, and when boiled with caustic potash and sulphate of copper remaining clear blue. It agrees with albumen and with mucin as I found it (though differing from it, as described by Eichwald) in giving a turbidity or slight precipitate with tannin, chloride of iron, and sulphate of copper. It differs from mucin in being insoluble in lime or baryta water, or in HCl of 10 per cent. Its most remarkable reaction is the change it undergoes by the addition of a very small quantity of caustic potash to the water in which it is suspended. It is then much more closely allied to mucin than to albumen. From the solubility and reactions of mucin being somewhat variable it is not improbable that, like albumen, it may occur in several forms of which this may be one; but its composition and relations must be determined by analyses, which I hope at a future period to be able to make.

LORIMER AND GILLIES, CLYDE STREET EDINBURGH.